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*Published in:*  
Microchimica Acta

*Link to article, DOI:*  
[10.1007/s00604-018-2999-8](https://doi.org/10.1007/s00604-018-2999-8)

*Publication date:*  
2018

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*  
Feng, X., Ashley, J., Zhou, T., & Sun, Y. (2018). Fluorometric determination of doxycycline based on the use of carbon quantum dots incorporated into a molecularly imprinted polymer. *Microchimica Acta*, 185(11), [500].  
<https://doi.org/10.1007/s00604-018-2999-8>

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**Fluorometric determination of doxycycline based on the use of carbon quantum dots  
incorporated into the molecularly imprinted polymer**

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**Keywords**

Nitrogen-doped carbon quantum dots, doxycycline, molecularly imprinted polymers, fluorescence quenching, pig serum

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## Abstract

A fluorometric assay is described for doxycycline detection. It is based on the use of nitrogen-doped carbon quantum dots (NCQDs) coated with molecularly imprinted polymers (MIPs). The NCQDs were prepared by a one-step hydrothermal reaction using citric acid and ethylenediamine (EDA) as the starting materials. Afterwards, the NCQDs were incorporated into the polymer that was molecularly imprinted with doxycycline. It is found that doxycycline quenched the fluorescence of the NCQDs, and that the functional groups on the surface of NCQDs play an important role in terms of quenching efficiency. A larger fraction of carboxyl groups presented on the surface of NCQDs leads to a higher quenching efficiency due to the enhanced electron transfer from NCQD to doxycycline. The NCQDs@MIPs composite can specifically and rapidly recognize doxycycline. Fluorescence drops linearly in the 5 to 50  $\mu\text{M}$  doxycycline concentration range, and the limit of detection is 87 nM. This method was successfully applied to the determination of doxycycline in spiked pig serum where it gave recovery rates of >94%.

## Introduction

In the last decades, carbon quantum dots (CQDs) have attracted a lot of attention due to their unique optical and electrical properties, such as narrow emission spectra, broad excitation spectra, and good photostability [1–3]. Compared with conventional inorganic semiconductor quantum dots containing heavy metals (e.g. Cd, Ag, Pb), CQDs exhibit high biocompatibility, hydrophilicity and have shown excellent performance in numerous applications[4–10]. For instance, the low toxicity makes them suitable for bioimaging and drug delivery[4–7]. The superior electrical properties of CQDs were utilized in photovoltaic devices[8–10]. Recently, substantial research has been devoted to using CQDs as fluorescent probes to detect different types of analytes, such as ions, small molecules, and biological macromolecules[2, 11–14]. This is because CQDs as the good electron donors/acceptors can induce the electron transfer with the analytes, resulting in fluorescence quenching. Depending on the starting materials, CQDs can present different functional groups on their surface, such as carboxyl groups, amino groups, and hydrogen groups, *etc.* Since the analytes differ in electron donor/acceptor groups, the CQDs should be tailor-made in order to achieve an optimal quenching effect. However, up to now, the effect of functional groups of CQDs on fluorescence quenching has seldom been studied.

Another challenge with CQD-based biosensors is the lack of selectivity, which makes it difficult to detect trace amounts of a specific analyte in complicated matrices. In order to improve the specificity, it is of great interest to combine molecularly imprinted polymers (MIPs) with CQDs [15–17]. The MIPs are prepared by the polymerization of the functional monomer, cross-linker, and initiator in the presence of a template. The subsequent removal of the template leaves cavities that are structurally and functionally complementary to the analyte [18]. Therefore, incorporating CQDs with MIPs can greatly enhance the selectivity of CQD-based biosensors.

Doxycycline is the most popular tetracycline derivative. It possesses anti-inflammatory activity for the treatment of infectious diseases and has been widely used as an additive to animal feeds due to its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria. However, relatively high levels of doxycycline residue in foodstuff can provoke side-effects such as liver damage, allergic reactions, yellowing of teeth, and gastrointestinal disturbance [19–22]. In order to ensure food is safe for human consumption, the European Union (EU) has set the maximum residue limits (MRLs) for doxycycline as 0.1 mg kg<sup>-1</sup> in mussel, 0.3 mg kg<sup>-1</sup> in skin and fat, 0.3 mg kg<sup>-1</sup> in the liver, and 0.6 mg kg<sup>-1</sup> in the kidney, respectively [23]. To date, several techniques have been used for the analysis of doxycycline residues in food samples, such as high-performance liquid chromatography [24, 25], thin-layer chromatography [26, 27], capillary electrophoresis [28, 29], immunoassays (*e.g.* ELISA) [30, 31]. However, they are time-consuming, require complicated sample preparation and trained personnel for operation. Hence, it is important to develop simple and cost-effective analytical methods for the detection of doxycycline.

In this work, an eco-friendly fluorescent probe based on nitrogen-doped carbon quantum dots (NCQDs) and MIPs was developed to detect doxycycline in food samples. The NCQDs with strong blue fluorescence were synthesized by a facile one-pot hydrothermal approach using citric acid and ethylenediamine (EDA) as carbon and nitrogen sources, respectively. It was found that the functional groups (mainly carboxyl group) of synthesized NCQDs played an important role in fluorescence-based doxycycline detection. The optimized NCQDs were incorporated into MIP nanoparticles via precipitation polymerization, where NCQDs acted as the optical material and MIPs provided specific binding sites. The NCQD@MIPs were significantly quenched upon interaction with doxycycline, indicating NCQD@MIPs integrated the merits of the high sensitivity of NCQD and good selectivity of MIPs. Furthermore, the NCQD@MIPs were successfully applied for the determination of doxycycline in pig serum.

## Experimental

### Chemicals

Citric acid, ethylene diamine (EDA), 2,2'-Azobis(2-methylpropionitrile) (AIBN), Methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM), doxycycline, cloxacillin, spiramycin, tetracycline, amoxicillin, sodium hydroxide (NaOH), potassium hydrogen phthalate (HOOC<sub>6</sub>H<sub>4</sub>COOH), Potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>), potassium chloride (KCl), hydrochloride acid (HCl), sodium tetraborate decahydrate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O), acetonitrile (ACN), methanol, acetone, porcine serum were purchased from Sigma-Aldrich (DK) (<https://www.sigmaaldrich.com/denmark.html>). Inhibitors were removed from all monomers prior to polymerization using pre-packed columns from Sigma Aldrich (<https://www.sigmaaldrich.com/denmark.html>). Q-Max® Cellulose acetate (CA) syringe filters (0.22 µm) were from FRISENETTE (DK) (<https://frisenette.dk/>). Nunclon 96-well flat-bottom transparent microwell plates and Nunclon 96-well flat-bottom black microwell plates were purchased from Thermo Scientific (DK) (<http://www.thermofisher.com/dk/en/home.html>). All chemicals were analytical or HPLC grade and were used without further purification.

### Apparatus

Fourier transform infrared (FT-IR) spectra were measured on a Spectrum100 (Perkin Elmer, MA, USA). X-ray photoelectron spectroscopy (XPS) analysis was examined by Thermo Scientific™ K-Alpha+™ X-ray photoelectron spectrometer system (Thermo Fisher Scientific, MA, USA). UV-Vis absorption and photoluminescence (PL) emission were performed using a Spark® multimode microplate reader (Tecan, Sweden). All transmission electron microscopy (TEM) images were carried out on a Tecnai G20 FEG (FEI, Oregon, USA) with an accelerating voltage of 200 kV. All

scanning electron microscopy (SEM) images were taken by a Quanta FEG 200 ESEM scanning electron microscopy (FEI, Oregon, USA).

### **Synthesis of NCQDs**

The NCQDs were prepared by a hydrothermal reaction using citric acid and EDA as starting materials. Briefly, 4 g of citric acid were mixed with different amounts of EDA (10 g, 5 g, 2.5 g, 1.25 g, 0.63 g, 0.31g, and 0.16 g) in 40 mL of DI water. The solution was sonicated for 30 min to ensure uniform dispersion prior to transfer to a PPL-lined autoclave. Next, the PPL-lined autoclave was put into a furnace and kept at 220 °C for 5 h. After the reaction, the product was cooled down to room temperature and filtered through a 0.22 µm CA syringe filter. Afterward, the resultant solution was dialyzed against pure water for 24 h. Finally, the NCQDs were dried using a freeze dryer and the obtained solid samples were stored at 4 °C in a dark environment for further studies.

### **Optimization of the functional groups on NCQDs and study of the quenching mechanism**

In order to optimize the degree of functionalization on NCQDs, 100 µL of NCQDs (2 µg mL<sup>-1</sup>) were mixed with 100 µL of doxycycline (200 µg mL<sup>-1</sup>) in phosphate buffered solution (pH 7). After overnight incubation, the fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark® multimode microplate reader. The quenching of the fluorescence signal was determined by comparing the fluorescence signals to the blank sample (only containing buffer and NCQD).

In order to study the quenching mechanism between NCQDs and doxycycline, 100 µL of NCQD\_7 (2 µg mL<sup>-1</sup>) was mixed with 100 µL of different concentrations of doxycycline (10 µg mL<sup>-1</sup>- 60 µg mL<sup>-1</sup>) in the phosphate buffered solution (pH 7) at 25°C, 35°C, and 50°C, respectively. After

overnight incubation, the fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark® multimode microplate reader. The UV/Vis spectra of NCQD, doxycycline, and NCQD@doxycycline were also recorded.

### **Synthesis of NCQD@MIPs**

The NCQD@MIPs was synthesized via a precipitation polymerization. Firstly, 0.25 mmol of doxycycline, 1.5 mmol MAA, and 2.78 mmol TRIM were dissolved in a 32.5 mL of mixed solvent of methanol and ACN (v/v, 4:9). The mixture was purged by nitrogen stream under the sonication for 30 min. Then, 0.5 mL (80 mg mL<sup>-1</sup>) of NCQDs was added dropwise into the mixture under the sonication to form a homogeneous solution. Prior to initiating the reaction, AIBN was added and the mixture was purged with nitrogen for 5 min. Then, the glass flask was removed from the sonication bath, sealed and heated at 60 °C for 16 h. After the reaction, the polymerized particles were collected by centrifugation at 4000 × g for 10 min. Doxycycline was extracted by a Soxhlet extraction apparatus using a solvent mixture of acetic acid and menthol (v / v, 1: 9) for 48 h and solvent was changed every 24 h. Next, the product was washed by MeOH several times to remove the residual acetic acid. Finally, the NCQD@MIPs was dried under a vacuum overnight. The non-imprinted polymer was prepared in the same manner except doxycycline was omitted during the polymerization reaction

### **Optimization of the binding conditions**

In order to study the effect of pH on quenching efficiency of NCQD@MIPs towards doxycycline, 100 µL of 1 mg mL<sup>-1</sup> NCQD@MIPs was mixed with 100 µL of 39 µM doxycycline in the pH range of 2 to 10. After overnight incubation, the fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark®



multimode microplate reader. The control experiments containing NCQD@NIPs were performed in the same manner as for the NCQD@MIPs. The experiments were conducted in duplicate.

In order to investigate the effect of incubation time on quenching efficiency of NCQD@MIPs towards doxycycline, 100  $\mu\text{L}$  of 1  $\text{mg mL}^{-1}$  NCQD@MIPs was incubated with 100  $\mu\text{L}$  of 39  $\mu\text{M}$  doxycycline solution at pH 6 at different time intervals (1-20 min). The fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark® multimode microplate reader. The control experiments containing NCQD@NIPs were performed in the same manner as for the NCQD@MIPs. The experiments were conducted in duplicate.

#### **Sensitivity and selectivity of NCQD@MIPs for doxycycline detection**

In order to study the sensitivity of NCQD@MIPs towards doxycycline, 100  $\mu\text{L}$  of 1  $\text{mg mL}^{-1}$  NCQD@MIPs (pH 6) was added to each well of a Nunclon 96-well flat-bottom black microplate. Next, 100  $\mu\text{L}$  of different concentrations of doxycycline (4.87  $\mu\text{M}$ , 9.75  $\mu\text{M}$ , 19.50  $\mu\text{M}$ , 39.00  $\mu\text{M}$ , 48.74  $\mu\text{M}$ ) in phosphate buffered solution (pH 6) was added and incubated for 15 mins. In order to study the selectivity of NCQD@MIPs towards doxycycline, 100  $\mu\text{L}$  of 1  $\text{mg mL}^{-1}$  NCQD@MIPs (phosphate buffered solution, pH 6) was added to each well of a Nunclon 96-well flat-bottom black microplate followed by adding 100  $\mu\text{L}$  of 39  $\mu\text{M}$  of doxycycline and its analog (i.e. cloxacillin, spiramycin, tetracycline, and amoxicillin). After 15 min incubation, the fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark® multimode microplate reader. The control experiments containing NCQD@NIPs were performed in the same manner as for the NCQD@MIPs. The experiments were conducted in duplicate.

## Real Sample Analysis

0.1 M  $\text{HOCC}_6\text{H}_4\text{COOH-HCl}$  buffer was added to the 20 mL pig serum to adjust the pH to 6. Afterwards, the adjusted pH serum was centrifuged at  $1000 \times g$  to remove potential aggregates. 100  $\mu\text{L}$  of  $1 \text{ mg mL}^{-1}$  NCQD@MIPs (phosphate buffered solution, pH 6) was mixed with 100  $\mu\text{L}$  of different concentrations of doxycycline spiked pre-treatment pig serum (0  $\mu\text{M}$ , 8.80  $\mu\text{M}$ , 15.60  $\mu\text{M}$ , 23.40  $\mu\text{M}$ ) into each well of a Nunclon 96-well flat-bottom black microplate. After 15 min, the fluorescent measurements were carried out with an excitation wavelength of 360 nm and the emission wavelength of 445 nm, using a Spark® multimode microplate reader. The concentration of doxycycline in the spiked pig serum was derived from the calibration curve and the sample recovery rate was determined by comparing the concentration of the measured result to the known concentration of the spiked sample. The experiments were conducted in triplicate.

## Results and discussion

### The effect of the functional groups of NCQDs on fluorescence quenching

So far, graphene-based CQDs often suffer from complicated preparation process and require secondary modification to obtain fluorescence [32, 33]. Non-element doped CQDs usually have low fluorescence quantum yield (QY) (< 10%) [34]. Therefore, NCQD with easy preparation process and high QY is more advantageous as the fluorescence probe. The NCQDs were prepared by the hydrothermal reaction as illustrated in Fig. 1, where citric acid acted as a carbon source to facilitate the dehydration and carbonization, and EDA acted as a nitrogen-containing precursor and surface passivation agent to enhance the fluorescent intensity of CQDs. The doxycycline can be quantified by fluorescence quenching of NCQDs due to the electron transfer from NCQD (donor) to the doxycycline (acceptor).

To thoroughly investigate the effects of functional groups of NCQDs on fluorescence quenching, we synthesized seven batches of NCQDs with different molar ratios of citric acid and EDA (**Table S1**). It can be seen that, from NCQD\_1 to NCQD\_7, the excitation-dependent behavior (*i.e.*, the emission spectra shifts with excitation wavelengths) became more obvious when a lower amount of EDA was added (Fig. S2). This was due to the passivation effect of the amino acid groups. It has been reported by Li et al. [35] that the excitation-dependent behavior can only be observed in NCQDs that not containing amino groups. In case of amino-rich NCQDs, the surface is fully covered by amino acid groups and the emission can only take place through the radiative transition of the carbogenic core. Therefore, the emission spectra were independent of the excitation wavelength [22]. Structural information of different NCQDs was also investigated by FT-IR spectroscopy. As shown in **Fig. 2a**, the bands at 3348  $\text{cm}^{-1}$ , 2936  $\text{cm}^{-1}$ , and 1652  $\text{cm}^{-1}$  were attributed to O-H, C-H, and C=O stretching, respectively. The band at 1543  $\text{cm}^{-1}$  was assigned to the bending of N-H and the band at 1168  $\text{cm}^{-1}$  was attributed to the stretching of the C-O-C functional groups. The peak ratio of COOH : NH<sub>2</sub> was increased when a decreased amount of EDA was used in the reaction. It is interesting to note that, when the added amount of EDA was less than 1.25 g, C-O-C groups start to appear. This may be attributed to a decreased coverage of amino acid groups on the surface of NCQDs so that the inner layer of C-O-C starts to be exposed to the outer layer.

Different batches of NCQDs were incubated with the same concentration of doxycycline. The fluorescence quenching effects are shown in **Fig. 2b**. The fluorescence intensity ratio ( $F_0 / F$ ) of NCQDs gradually increased with an increasing molar ratio of COOH : NH<sub>2</sub> to NCQDs, where  $F_0$  and  $F$  present the fluorescent intensities of NCQDs in the absence and presence of the target

molecule doxycycline respectively. The result shows that the carboxyl groups on NCQDs helped to enhance fluorescence quenching. The fluorescent quenching mechanism was determined by investigating UV/Vis absorption behavior and temperature-dependent behavior. In general, the fluorescent quenching mechanism can be divided into two types, namely dynamic quenching, and static quenching. Dynamic quenching occurs when random collisions of small molecules deactivate the excited state of the fluorophore. During this process, the chemical change does not happen. Therefore, this does not affect the UV/Vis absorption spectra of the molecules. Since the dynamic quenching based on the molecular collisions, quenching is enhanced by increasing temperature. On the contrary, for the static quenching, a fluorophore forms a non-fluorescence complex with the small molecule, upon which a chemical change occurs. As shown in **Fig. 2c**, the absorption peaks of the UV/Vis spectrum for NCQD@doxycycline were in the same position compared with the overlapped spectrum of NCQDs and doxycycline, indicating the quenching was dynamic. The mechanism was also confirmed by the temperature-dependent behavior. The  $F_0/F$  was plotted versus doxycycline concentration at different temperatures as shown in **Fig. 2d**. The slope of the Stern-Volmer plot increased with the temperature, which was due to the increased frequency of collisions between doxycycline and NCQDs at higher temperatures.

Since the interactions between NCQDs and doxycycline follow a dynamic quenching mechanism, the quenching efficiency depends on the dipole-dipole interaction between donor and acceptor. In this case, the -COOH groups present on the surface of NCQD may form hydrogen bonds with -OH as well as -NH<sub>2</sub> groups on the doxycycline [36], which would provide a better alignment of the donor and acceptor centers, thus improving the coupling and quenching. Therefore, to obtain the best sensitivity, the NCQDs with the highest amount of carboxyl group (NCQD\_7) was chosen for all future experiments.

## Quenching Efficiency of NCQD@MIPs for the Determination of Doxycycline

The following parameters were optimized prior to investigating quenching efficiency of NCQD@MIPs for the determination of doxycycline: (a) sample pH value; (b) incubation time. Respective data and Figures are given in the Electronic Supporting Material (Figure S3). It was found that at pH 6 and incubation time of 15 minutes, maximum fluorescence quenching was obtained. Under the selected optimal conditions, the sensitivity of NCQD@MIPs towards doxycycline was studied. Although both NCQD@MIPs and NCQD@NIPs showed fluorescence quenching towards doxycycline, it can be clearly seen that the fluorescence intensity of NCQD@MIPs decreased notably in the presence of increasing concentrations of doxycycline (Fig. 3a), NCQD@NIPs only showed a slight decrease under the same concentrations of doxycycline (Fig. 3b). The fluorescence quenching was plotted against the concentration of doxycycline using the Stern-Volmer relationship [37]:

$$F_0/F = 1 + K_{sv} [c] \quad (1)$$

Where  $F_0$  and  $F$  present the fluorescent intensities of NCQD@MIPs in the absence and present of the target molecule doxycycline respectively,  $K_{sv}$  is the Stern-Volmer quenching constant of target molecule doxycycline and  $[c]$  is the concentration of doxycycline. As shown in Fig. 3c, both NCQD@MIPs and NCQD@NIPs fit the linear relationship in the range of 5-50  $\mu$ M. The regression equations were:

$$\text{NCQD@MIPs: } F_0/F = 1 + 0.01042 C_{\text{Doxycycline, } \mu\text{M}}, (R^2=0.99665) \quad (2)$$

$$\text{NCQD@NIPs: } F_0/F = 1 + 0.00298 C_{\text{Doxycycline, } \mu\text{M}}, (R^2=0.99051) \quad (3)$$

Moreover, the imprinting factor (IF) is defined by the ratio between  $K_{sv, \text{ MIP}}$  and  $K_{sv, \text{ NIP}}$ . Under optimized conditions, the IF was calculated to be 3.5, suggesting that NCQD@MIPs can be used to

selectively recognize the doxycycline. This high IF value is due to the specific recognition of doxycycline to NCQD@MIPs via hydrogen bonding, resulting in an increased degree of fluorescent quenching caused by electron transfer from the NCQDs to doxycycline. In addition, the limit of detection (LOD) of this probe was 86.9 nM (38.6  $\mu\text{g} / \text{kg}$ ) which was calculated by  $3\delta / K_{\text{sv, MIP}}$ . This value is far below the MRLs of doxycycline set by EU. Compared with other methods that were reported in the literature (**Table 1**), our NCQD@MIPs demonstrated comparable or higher sensitivity.

One potential limitation of the NCQD@MIPs-based method is that some analytes emit fluorescence at similar wavelengths as CQDs when exposed to UV, which interferes with the detection. In such cases, blue NCQDs can be changed to green or red NCQDs to avoid overlap in fluorescence emission spectra.

### Selectivity of MIP@NCQD for Doxycycline

Doxycycline and its analog (i.e. cloxacillin, spiramycin, tetracycline, and amoxicillin) were chosen to investigate the selectivity of MIP@NCQD towards doxycycline. The structures of doxycycline and its analog are shown in **Fig. 4a**. It can be seen in **Fig. 4b**, NCQD@MIPs gave the highest response to doxycycline compared to the other antibiotics, indicating the imprinted cavities complemented the shape, size, and functional groups of doxycycline. Other antibiotics caused slight fluorescent quenching owing to their physical adsorption on the surface of NCQD@MIPs. In addition, the NCQD@MIPs also exhibited a significant increase in quenching when compared to the corresponding NCQD@NIPs.

### Detection of doxycycline in serum samples

Finally, the optimized method was applied to detect doxycycline in pig serum. Doxycycline was spiked into pig serum with five different concentrations. The results are summarized in **Table 2**. The concentrations of the doxycycline were determined by interpolation of the quenched fluorescence signal using the Stern-Volmer calibration plot. No response was observed when 0  $\mu\text{M}$  doxycycline was added into pig serum, indicating that the matrix effects had minimal interference on the analytical results. The recovery rates of doxycycline in the pig serum were obtained in the range of 94.68 – 104.04% with RSD (n=3) less than 4.50%. It can be seen that our method endowed a good accuracy and low detection limit with minimal sample pretreatment, which is very promising for applications in real samples.

## Conclusion

In summary, a fluorescent **probe** based on NCQDs and MIPs was developed for the detection of doxycycline. The functional groups on the surface of NCQDs **were** optimized to obtain maximum fluorescence quenching efficiency. The NCQDs were incorporated into MIP microspheres, allowing for the detection of trace amounts of doxycycline with both high sensitivity and high specificity. **Finally, the fluorescent probe was successfully used to determine doxycycline in real serum sample with the low detection limit and high recovery rate, which revealed its great potential in advanced analytical applications.**

## Acknowledgment

This work was financially supported by the Villum Fonden, Denmark, Project No. 13153.

## Compliance with ethical standards

The authors declare no competing financial interest.

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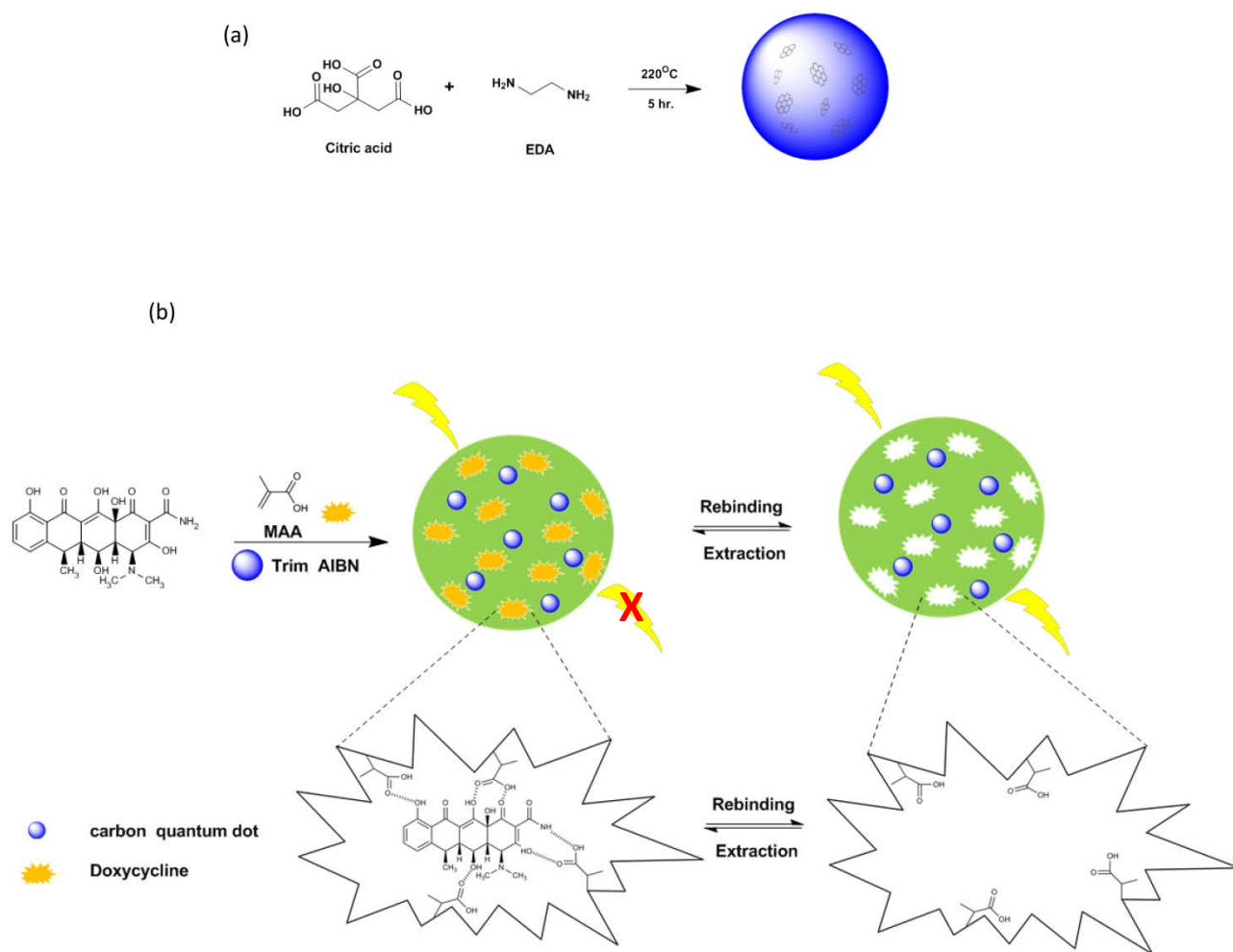
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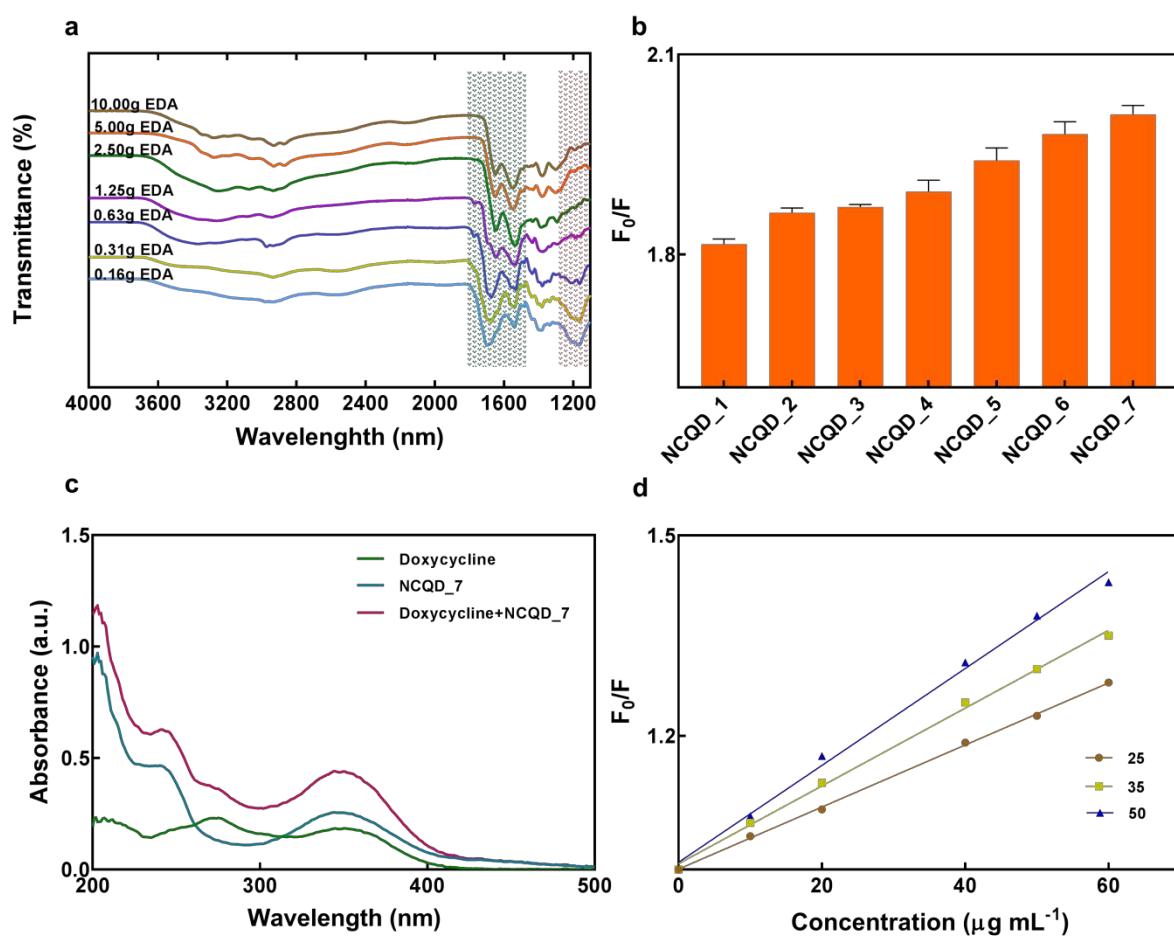
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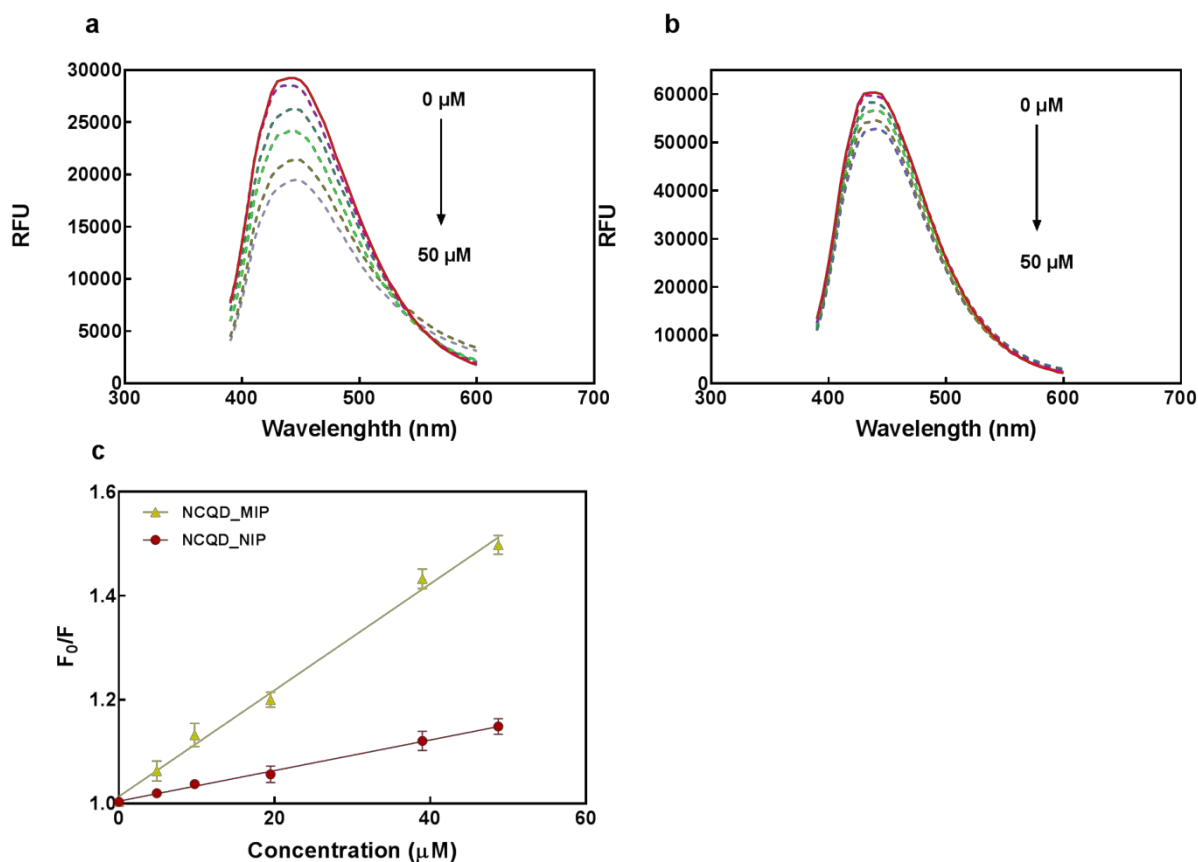
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**Fig. 1** (a) Synthesis of NCQDs using citric acid and EDA as starting materials. (b) Schematic illustration for the synthesis of NCQD@MIPs.

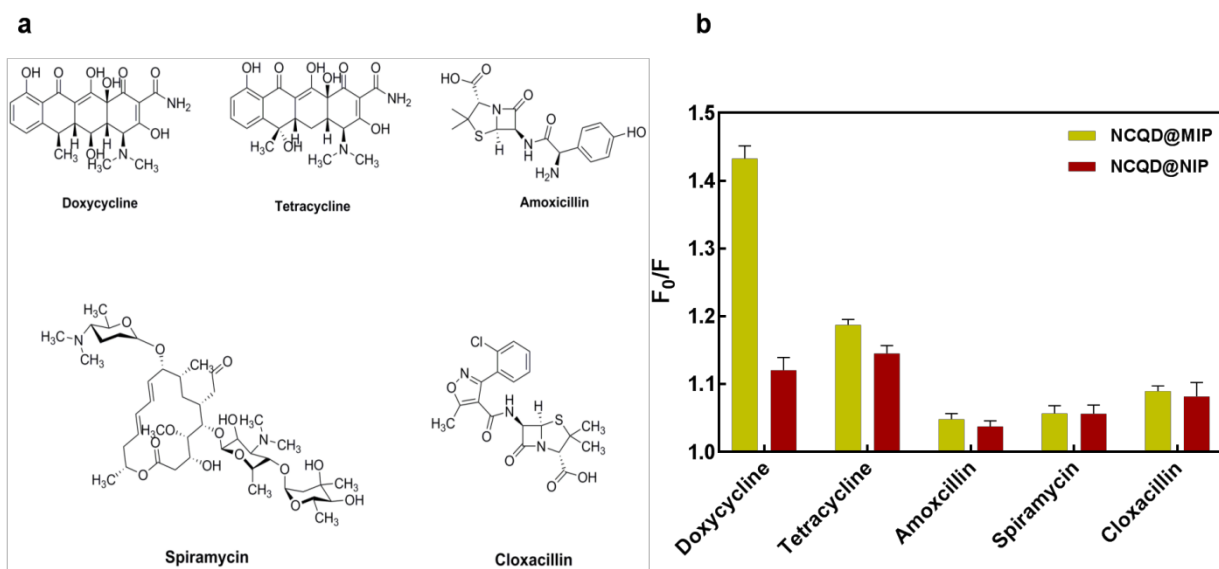


**Fig. 2** (a) FT-IR spectra of different NCQDs; (b) Effect of carboxylic group of NCQDs on fluorescence quenching efficiency ( $F_0$  and  $F$  present the fluorescent intensities of NCQD in the absence and present of the target molecule doxycycline, respectively); (c) UV/Vis spectrum of doxycycline, NCQD\_7, and the mixture of doxycycline and NCQD\_7; (d) Stern-Volmer plot of NCQD\_7 towards doxycycline at 25°C, 35°C, and 50°C.



**Fig. 3** (a) and (b) are fluorescence spectra of NCQD@MIPs and NCQD@NIPs when incubated with different concentrations of doxycycline (4.87  $\mu\text{M}$ , 9.75  $\mu\text{M}$ , 19.50  $\mu\text{M}$ , 39.00  $\mu\text{M}$ , 48.74  $\mu\text{M}$ ) at phosphate buffered solution pH 6, respectively. The spectra were excited at the wavelength of 360 nm (c) The Stern-Volmer plots of NCQD@MIPs (1 mg mL<sup>-1</sup>) and NCQD@NIPs (1 mg mL<sup>-1</sup>) when incubated with different concentrations of doxycycline at phosphate buffered solution (pH 6). Experiments were performed in duplicate.





**Fig. 4** (a) Structure of doxycycline and its analog; (b) Selectivity of 100  $\mu\text{L}$  of 1  $\text{mg mL}^{-1}$  NCQD@MIPs and NCQD@NIPs towards 100  $\mu\text{L}$  of 39  $\mu\text{M}$  of doxycycline and its analog (i.e. cloxacillin, spiramycin, tetracycline, and amoxicillin) at phosphate buffered solution (pH 6). Experiments were performed in duplicate.

**Table 1. Comparison of different methods for detection of doxycycline.**

Analytical Method	Analytical ranges ( $\mu\text{M}$ )	LODs (nM)	References
HPLC	56.28-4502.59	2229	[24]
ELISA	Not given	3.9	[30]
Capillary electrophoresis	0.056-0.39	36.3	[29]
Spectrophotometric method	17.5-348	712	[38]
FTIC@MIPs by core-shell polymerization	0.2-6	117	[39]
TGA-capped CdTe quantum dot	1.9-61	110	[36]
NCQD@MIPs by precipitation polymerization	5-50	86.9	This work

**Table 2. Recovery of doxycycline in pig serum (pH 6). (n=3)**

Sample	Spiked ( $\mu\text{M}$ )	Found( $\mu\text{M}$ )	Recovery (%)	RSD (%)
1	0	ND	-	-
2	29.24	30.42	104.04	1.80
3	23.40	22.55	96.17	3.96
4	15.60	14.77	94.68	2.87
5	8.80	8.64	98.18	4.50